Iranian Journal of Veterinary Science and Technology

Received: 2018- Dec-19
Accepted after revision: 2019- May-27
Published online: 2020- Feb- 12

Short Communication

DOI: 10.22067/veterinary.v11i2.77631

Molecular detection of mouse hepatitis virus in laboratory mouse colonies

Roozbeh Fallahi[a], Fatemeh Abedini[a], Gholam Reza Shokri[b]

[a] Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.

[b] Research and Development Laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

ABSTRACT

The animal health monitoring is required to issue health certificates. The viral hepatitis virus is one of the most important infectious agents in mice breeding colonies. This research used RT-PCR to identify contaminations to mouse hepatitis virus. 18 out of 29 specimens were found to be infected, a prevalence of 62%. PCR product was purified and sequenced. Phylogenetic analysis revealed that the identified strain in this study was closely related to a strain reported from France. In the conventional system, contamination with different infectious agents is inevitable, thus it is better to replace the contaminated colonies with clean animals.

Keywords

Molecular detection, Mouse, Hepatitis virus

Abbreviations

MHV: Mouse hepatitis virus
ELISA: Enzyme linked immunosorbent assay
RT-PCR: Reverse transcription-polymerase chain reaction
NIH: National Institutes of Health
NC: Nucleocapsid
FELASA: Federation of European Laboratory Animal Science Associations
IVC: Individually ventilated cage

Corresponding author:
Roozbeh Fallahi
Tell/Fax: 02634 502805
Email: fallahiroozbeh@gmail.com

Mouse hepatitis virus is a common infection in a large number of laboratory mouse colonies and is known to interfere with research results (1). MHV is an enveloped virus which has a 31Kb single-strand positive RNA genome. MHV belongs to the Coronaviridae family and replicates in the cytoplasm of infected cells using a viral RNA-dependent RNA polymerase which is translated from the genomic RNA (2, 3). MHV strains are classified as respiratory tropic or enterotropic groups based on tissue distribution of primary infection (2, 4), although the enterotropic infection is considered to be the most common from of infection (10). MHV is well known to be the most common virus of laboratory mice (2, 5). Natural infections with MHV remain widespread in most laboratory mouse populations despite the efforts to detect and eradicate this agent (6). Current data based on serological tests estimate that 60 to 80% of laboratory animal colonies are infected with MHV (2). Since its first description by Cheever in the late 1940's, MHV has been shown to alter the results of in vivo experiments using other infectious and non-infectious agents (2, 7). Concomitant infection with MHV has been correlated with altered responses to tumours (8) and to other viruses (2). Also, immune system-modulation experiments were noted to potentiate MHV infection and disease (2, 7). MHV is able to spread rapidly in mouse colonies because of its high contagiousness (2, 9, 10). Therefore an early detection of MHV infection is very important. Current methods which are used to detect MHV infection include ELISA and immunofluorescence techniques. The diagnosis of MHV infection is mainly performed by serological assays due to the difficulties in finding histological lesions and in isolating the virus in tissue culture (2, 11). However, the seroconversion of the animal sentinels or the newly infected ones requires a waiting period before a serologic assay can be used. The direct detection of viral nucleic acid using molecular bio1ogy methods in clinical or necropsy specimens would be a quick and powerful means to detect an outbreak or a sub-clinical condition affecting the animals (2, 11-14). RT-PCR has been effective in the detection of MHV in tissues and feces of infected mice (10, 12, 22). The aim of this study was to evaluate mouse hepatitis virus in NIH mice colonies in one laboratory animal facility in Iran using RT-PCR method.

According to the FELASA instruction, taking into account a 10% prevalence of contamination and 95% confidence, 29 samples was needed. In this study, NIH breeding mice from both sexes in the breeding room were randomly selected and monitored for mouse hepatitis virus according to the ethical protocols. Samples were collected from the intestine (colon) containing feces and prepared by standard methods. Then, RT-PCR was performed (10, 11, 12, 22). The sequence of NC gene (F: 5'- CAGCAGTGTTTTGGAAAGAGAG-3', R:5'- TGGGCTTTGCAACGCTTA-3')(2) available in the Genbank (Accession number, EMBL: AB551247.1) were cloned in pUC57 vector (CinnaGen, Tehran, Iran). The pUC57-NC plasmid was used as positive control. Plasmid extraction was performed by the GF-1 kit (Vivantis, Malaysia) in accordance with the protocol. RNA was extracted from intestinal tissue samples using Trizol (25). Contaminating genomic DNA was removed by DNase I (Fermentas, ... treatment) (25). The conversion of RNA to cDNA was carried out using the Viva 2 steps RT-PCR kit (Vivantis, Malaysia) (25). The reaction was carried out with a final volume of 25 µl according to the protocol (26). The PCR reaction ncluded: initial denaturation at 94 °C for 5 minutes, 30 cycles f denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute, and extention at 72 °C for 1 minute, and a final extention at 72 °C for 10 minutes (26).

In 18 colon samples containing feces, the infection to MHV was positively detected. Therefore, the prevalence of this infection was calculated to be 62% (Figure 1).

For phylogenetic study, the positive sample was tested 3 times. Therefore, a PCR product was sequenced by BIONEER (South Korea). The alignment study was conducted through the EMBL-EBI and Klign (2.0) program and the sequence acquired from Sanger sequencing was compared with the sequences of the four other species obtained from the NCBI GenBank. In the phylogeny tree, the strain KX774640: 0.04601 belongs to this study and other species are X63538: 0.03507, L37760: 0.02799, L37759: 0.01186 and L37758: 0.02207, respectively. The most closely related strain in this study has been shown to be X63538: 0.03507 in France. The degree of affinity is found in the phylogeny tree (Figure 2).

Based on the recommendation of the FELASA, animal health monitoring is required to issue health certificates that are required for quality systems and quality control of production and research institutes (11, 15, 16). Many infectious agents in laboratory animals cause infections in humans and they are zoonoses (17, 18). Recommendations should be based on individual and local needs, considerations of research work, factors that are prevalent regionally, and national goals that are relevant in each country (11, 15, 16). The transmission of infectious agents and the presence of allergenic agents in open-cage systems are more prevalent than closed systems. Thus, it is much more important to carry out health monitoring programs in conventional open-cage systems. (11, 15, 16). In Iranian laboratory animal breeding centers, despite the advances made in design and breeding methods,

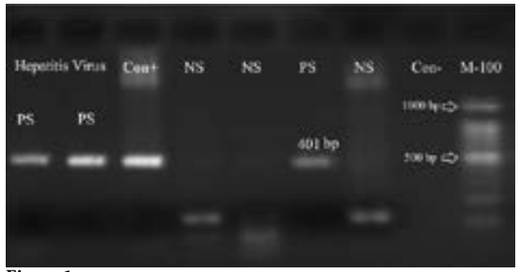

Figure 1
Agarose gel electrophoresis of RT-PCR products from Mouse Hepatitis Virus, NC gene. [M-100: PCRBIO Ladder IV DNA Marker- 100 bp (Arian Gene Gostar), Con-: Negative Control, Con+: Positive Control, NS: Negative Sample, PS: Positive Sample]

some infections, especially parasitic, bacterial and viral infections, are still present. Although clinical symptoms may not be seen in contamination with infectious agents, it can negatively affect the quality of the vaccine and biological products tested in these animals (11, 15).

In this study, the prevalence of this infection was 62%. Despite the advances made in the design and method of breeding centers especially in this center, the prevalence of this viral infection is still high. Although there are no clinical signs of contamination with these virus, it can negatively affect the results of the research and quality control tests. In the conventional system, contamination with different microbial agents is inevitable, but it is better to replace the contaminated colonies with clean animals. There are many reports on the health surveillance of viral infectious agents in foreign countries. The first description of the mouse hepatitis virus was provided by Cheever et al. (1949) (16). Parker (1979) identified 60-80% infection rates of mouse hepatitis virus in laboratory animals (17). Kagiyama et al. (1986) introduced the mouse hepatitis virus as one of the common viruses in laboratory mice (5). Homberger et al. (1991) and Yamada et al. (1993) introduced the RT-PCR as a suitable method for detecting the mouse hepatitis virus (18, 19). Yamada et al. (1993) announced that the virus could rapidly spread to the laboratory colonies due to easy transfer through contaminated materials (19). Adami et al. (1995)and Barthold and Smith (1990) published reports of viral hepatitis infection in mice and rats in both animal and wildlife animal breeding centers (3, 7). Jacoby and Lindsey (1997) reported the hepatitis mouse virus in 60% of the conventional breeding centers and 10% of the eligible systems of the barriers (20). Cecilio et al. (2000) detected mouse hepatitis virus by Nested PCR in liver tissue samples of laboratory mice (2). Matthaei et al. (1998) used the polymerase chain reaction to diagnose a natural outbreak of mouse hepatitis virus infection in nude mice (21). Oyanagi et al. (2004) detected the MHV-RNAs in mouse intestines and in filter dust in mouse room ventilation duct by a modified RT-nested PCR (22). Wang et al. (1999) diagnosed the mouse hepatitis virus contamination in nude mouse population by using RT-PCR (23). Nowadays, the large production and breeding centers are tested for the diagnosis of mouse hepatitis virus by PCR every six weeks (24). In Iran, there have been no investigations into this virus. Fallahi and Mansouri (2017) reported the health monitoring of NIH laboratory mice to Clostridium piliforme (24). The use of filter cages in the IVC breeding system prevents the transmission of airborne contamination. Although infection with the virus is unusual in humans, full compliance with health rules is required for staff working with rodents.

Acknowledgment

The author would like to thank the staff of Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute for their kind cooperation.

Author Contributions

RF prepared the experimental design, wrote and revised the article, and managed the research. FA performed the experiments.

Figure 2
The phylogenic tree of the strain detected in this research (KX774640:0.04601) As shown in the picture, the most closely related specie is X63538: 0.03507 from France.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Wood CS. Detection of mouse hepatitis virus: A thesis submitted for the degree of Master of Science in the University of Dundee; 2015.
2. Cecílio AB, Cândido AL, Resende M, Bontempo ED, Martins AdS. Detection of mouse hepatitis virus in mouse colonies using the nested polymerase chain reaction. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2000;52(4):307-12.
3. Adami C, Pooley J, Glomb J, Stecker E, Fazal F, Fleming JO, et al. Evolution of mouse hepatitis virus (MHV) during chronic infection: quasispecies nature of the persisting MHV RNA. Virology. 1995;209(2):337-46.
4. Compton SR, Barthold SW, Smith AL. The cellular and molecular pathogenesis of coronaviruses. Laboratory Animal Science. 1993;43(1):15-28.
5. Kagiyama N, Takakura A, Itoh T. A serological survey on 15 murine pathogens in mice and rats. Experimental Animals. 1986;35(4):531-6.
6. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Advances in virus research. 2011;81:85-164.
7. Barthold SW, Smith AL. Duration of mouse hepatitis virus infection: studies in immunocompetent and chemically immunosuppressed mice. Laboratory animal science. 1990;40(2):133-7.
8. Akimaru K, Stuhlmiller GM, Seigler H. Influence of mouse hepatitis virus on the growth of human melanoma in the peritoneal cavity of the athymic mouse. Journal of surgical oncology. 1981;17(4):327-39.
9. Percy DH, Barthold SW. Pathology of Laboratory Rodents and Rabbits: Blackwell Publishing Professional; 2007.
10. Yamada YK, Yabe M, Takimoto K, Nakayama K, Saitoh M. Application of nested polymerase chain reaction to detection of mouse hepatitis virus in fecal specimens during a natural outbreak in an immunodeficient mouse colony. Experimental animals. 1998;47(4):261-4.
11. Mahler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, et al. FELASA Recommendations for the Health Monitoring of Mouse, Rat, Hamster, Guinea Pig and Rabbit Colonies in Breeding and Experimental Units. Laboratory animals. 2014;48(3):178-92.
12. Casebolt DB, Qian B, Stephensen CB. Detection of enterotropic mouse hepatitis virus fecal excretion by polymerase chain reaction. Laboratory animal science. 1997;47(1):6-10.
13. Kunita S, Terada E, Goto K, Kagiyama N. Sequence analysis and molecular detection of mouse hepatitis virus using the polymerase chain reaction. Laboratory animal science. 1992;42(6):593-8.
14. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning. A Laboratory Manual2 Cold Spring Harbor Laboratory Press New York Google Scholar1989.
15. Ruggeri FM, Di Bartolo I, Ponterio E, Angeloni G, Trevisani M, Ostanello F. Zoonotic transmission of hepatitis E virus in industrialized countries. The new microbiologica. 2013;36(4):331-44.
16. Cheever FS, Daniels JB, Pappenheimer AM, Bailey OT. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin: I. Isolation and biological properties of the virus. The Journal of experimental medicine. 1949;90(3):181.
17. Parker J, editor possibilities and limitations of virus control in laboratory animals. Animal quality and models in biomedical research: 7th symposium of the International Council for Laboratory Animal Science (ICLAS), Utrecht, 21-23 August 1979/editors, A Spiegel, S Erichsen, HA Solleveld; 1980: Stuttgart, Gustav Fischer, 1980.
18. Homberger FR, Smith AL, Barthold SW. Detection of rodent coronaviruses in tissues and cell cultures by using polymerase chain reaction. Journal of clinical microbiology. 1991;29(12):2789-93.
19. Yamada YK, Yabe M, Yamada A, Taguchi F. Detection of mouse hepatitis virus by the polymerase chain reaction and its application to the rapid diagnosis of infection. Laboratory animal science. 1993;43(4):285-90.
20. Jacoby R, Lindsey J. Health care for research animals is essential and affordable. The FASEB journal. 1997;11(8):609-14.
21. Matthaei KI, Berry JR, France MP, Yeo C, Garcia-Aragon J, Russell PJ. Use of polymerase chain reaction to diagnose a natural outbreak of mouse hepatitis virus infection in nude mice. Laboratory animal science. 1998;48(2):137-44.
22. Oyanagi M, Kato A, Yamada YK, Sato NL. Detection of MHV-RNAs in mouse intestines and in filter dust in mouse room ventilation duct by a modified RT-nested PCR. Experimental animals. 2004;53(1):37-41.
23. Wang RF, Campbell WL, Cao WW, Colvert RM, Holland MA, Cerniglia CE. Diagnosis of mouse hepatitis virus contamination in mouse population by using nude mice and RT-PCR. Molecular and cellular probes. 1999;13(1):29-33.
24. Fallahi R, Mansouri MA, Health monitoring of Razi Institute laboratory mice (NIH strain) to Clostridium piliforme in 1395. Vet Res Biol Pro 2017; 117: 78-84.
25. Hubrecht RC, Kirkwood J. The UFAW handbook on the care and management of laboratory and other research animals: John Wiley & Sons; 2010.
26. Quintero-Spongberg E, Arny R, Company RRB. Forthcoming Books: R.R. Bowker Company; 1999.